

Ultrastructural changes of the mitochondria in autotransplanted canine pancreas after cold storage for 72 and 96 hours

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To investigate the limit of safe cold storage of the pancreas, the left lobe of the pancreas was stored in University of Wisconsin solution for 72 or 96 hours at 4°C and then autotransplanted in 10 mongrel dogs. Ultrastructural changes of the mitochondria in islet B cells, acinar cells, and endothelial cells were studied. After storage for 72 hours, the mitochondria of islet B cells were well preserved. After 96 hours, however, mitochondrial swelling was evident as well as a decrease in the number of cristae. These changes were more severe in acinar cells than in islet cells, but they were normalized in both types of cells within 6 hours of autotransplantation. Three of the 5 grafts stored for 96 hours showed long-term function and maintained a normal fasting plasma glucose level. However, impairment of glucose tolerance following intravenous glucose loading was significant when compared to the 72 hours storage group. Three grafts stored for 96 hours failed to function due to arterial thrombosis, and degeneration and necrosis of the islet endothelial cells was demonstrated by electron microscopy. This endothelial cell damage was possibly associated with impaired glucose tolerance and arterial thrombosis in the 96 hours group. These findings suggest that pancreatic storage for 72 hours has little effect on graft viability or function, while storage for 96 hours causes definite impairment.

Key Words: Canine pancreatic transplantation, University of Wisconsin solution, Pancreatic preservation, Mitochondria

Introduction

Prolonged preservation of the pancreas has become possible since the development of University of Wisconsin (UW) solution by Wahlberg *et al.* (1). Simple cold storage preservation of the canine pancreas for 72 hours has been achieved (2), a preliminary study of preservation for 96 hours has been reported (3). Although the morphological changes during cold storage have been reported (4-6), chronological documentation of the changes occurring after transplantation of the stored pancreas is rare. In the present study, to determine the limit of safe cold storage of the

pancreas, we observed the ultrastructural changes of the pancreatic mitochondria after cold storage in UW solution for 72 or 96 hours as well as after autotransplantation in dogs. In addition, we investigated glucose metabolism and survival after the autotransplantation of preserved pancreatic grafts.

Materials and Methods

Animals.

Ten mongrel dogs of both sexes weighing 8-16kg were used. Operations were done under inhalational anesthesia (gas-oxygen-fluothane) following endotracheal intubation under intravenous pentobarbital (25mg/kg).

Pancreatic preservation and Autotransplantation.

After removal of the spleen, the splenic artery and vein were dissected out and ligated proximally to allow removal of the left lobe of the pancreas. The resected lobe was flushed with 60-100ml of cold UW solution (Du Pont Japan LTD), followed by perfusion with a further 200ml of cold UW solution. Cold storage at 4°C was continued for 72 hours or 96 hours (n = 5 each). At the time of pancreatic resection, a silicon stent tube (2mm in diameter) was inserted into the pancreatic duct and the stump of the pancreas was closed with a mattress suture. After storage for 72 or 96 hours, the preserved pancreatic lobes were autotransplanted into the right iliac fossa. Arterial reconstruction was done by an end-to-end anastomosis between the splenic artery and the common iliac artery using 6-0 nylon, after which an end-to-side venous anastomosis was done between the splenic and common iliac veins. The pancreatic duct was anastomosed to the urinary bladder. Then the remaining right lobe of pancreas was completely resected by Markowitz's technique (7), which left the duodenum intact.

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Investigation of endocrine function after autotransplantation.

All the dogs were fasted for 3 days after autotransplantation, and were given 1,000ml/day of lactated Ringer's solution with 1g/day of penicillin during this period. Oral intake was started from day 4 after surgery.

Fasting plasma glucose levels were measured every day for two weeks after transplantation, and once a week subsequently. An intravenous glucose tolerance test employing a load of 0.5g/kg of glucose was carried out on day 7 after autotransplantation. Plasma glucose levels were measured by the glucose oxidase method, and the normal fasting glucose level was defined as below 150mg/dl. The percent decrease in the plasma glucose concentration per minute (k-value) was calculated from the glucose concentrations obtained at 10 to 60min (8).

Morphological studies.

For electron microscopy, pancreatic biopsy specimens were obtained after cold storage for 72 or 96 hours and at 1, 3, and 6 hours after autotransplantation. The specimens were immediately fixed in 2% glutaraldehyde and then in 2% osmium, after which they were dehydrated with ethanol and embedded in Epon 812. Sections were stained with toluidine blue for microscopic examination. Ultrathin sections were cut, stained with uranyl acetate, and observed with a JEM-1200EX electron microscope (Nippon Denshi, Tokyo, Japan).

Statistical analysis.

Results are given as the mean \pm standard deviation (SD). Student's t-test was used for statistical analysis, and $p < 0.05$ was considered to indicate a significant difference.

Results

Survivals and pancreatic endocrine function.

After transplantation, fasting plasma glucose levels remained normal for 1 week in all 5 dogs given pancreatic grafts preserved for 72 hours and in 4 of the 5 dogs in the 96 hours group. The K value of the 72 hours group was $2.2 \pm 0.73\%/min$, and that of the 4 dogs in the 96 hours group was significantly lower $0.95 \pm 0.27\%/min$ ($p < 0.05$ vs. the 72 hours group) (Table 1).

Two dogs (nos. 1 and 2) in the 72 hours group died of pancreatitis on days 13 and 15 after autotransplantation. However, normal fasting plasma glucose levels were maintained in three dogs (nos. 3-5) until death from debility on days 96, 122, and 169 after autotransplantation. Three dogs (nos. 6, 8 and 9) in the 96 hours group were sacrificed on

Table 1. Pancreatic function after transplantation following cold storage in UW solution for 72h (Group I) or 96 h (Group II).

Dog No.	K value (day 7)	Remarks
Group I		
1	1.1	Dead day 13, pancreatitis
2	1.8	Dead day 15, pancreatitis
3	2.8	Dead day 96, debility
4	2.4	Dead day 122, debility
5	2.8	Alive day 169, good function
mean \pm SD	$2.2 \pm 0.73^*$	
	K value (day 7)	Remarks
Group II		
6	-	Sacrificed day 7, thrombosis
7	0.6	Dead day 8, pancreatitis
8	1.2	Sacrificed day 14, thrombosis
9	1.1	Sacrificed day 15, thrombosis
10	0.9	Alive day 42, good function
mean \pm SD	$0.95 \pm 0.27^*$	*P < 0.05

K value: Percent decrease in the plasma glucose concentration per minute during the intravenous glucose tolerance test (loading dose of glucose: 0.5g/kg).

days 7, 14, and 15 after autotransplantation, respectively, because of severe hyperglycemia. In these animals, post-mortem examination showed evidence of arterial thrombosis in the grafts. In one dog (no. 7), hemorrhagic pancreatitis caused death on day 8 after transplantation. In contrast, dog no. 10 survived for 42 days after autotransplantation with normal fasting plasma glucose levels (Table 1).

Macroscopic and histologic findings.

Slight edema was observed after preservation of the pancreas for 96 hours, but was not found in the 72 hours group. The pancreatic parenchyma became greyish white during cold storage, but returned to a normal color after the reconstitution of blood flow. In both groups, pancreatic juice began to flow soon after the completion of vascularreconstruction.

Light microscopy revealed diffuse vacuolation and degeneration of the acinar cells in the 72 hours group, while a marked decrease of zymogen granules and diffuse vacuolation were noted in the 96 hours group. However, an increase of zymogen granules was noted even 3 hours after autotransplantation.

Electron microscopic findings.

Electron microscopy indicated that pancreatic islet B cells were almost normal in the 72 hours group (Fig. 1a). Although the number of cristae was slightly decreased in the mitochondria, their arrangement was regular. At 6 hours after transplantation, the number of mitochondria and

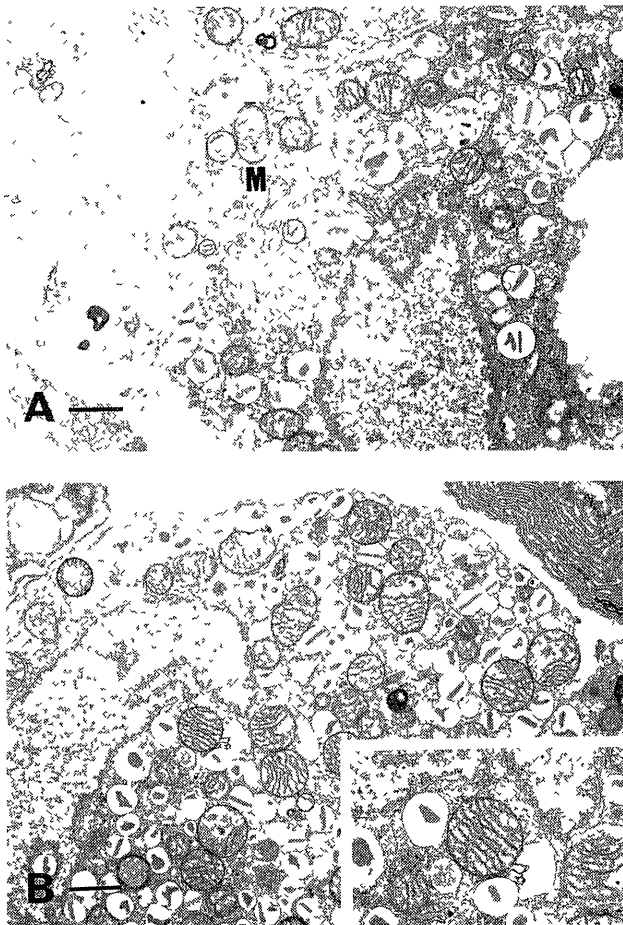


Fig. 1. (A) Electron micrograph of B cells in a pancreatic graft stored for 72h at 4 °C in UW solution (Group I) There is no swelling of the mitochondria [M] and only slight loss of the cristae $\times 6,000$ (B) The B cells of the same grafts at 6h after transplantation $\times 6,000$ Inset The number of cristae has increased and their arrangement is more regular $\times 12,000$

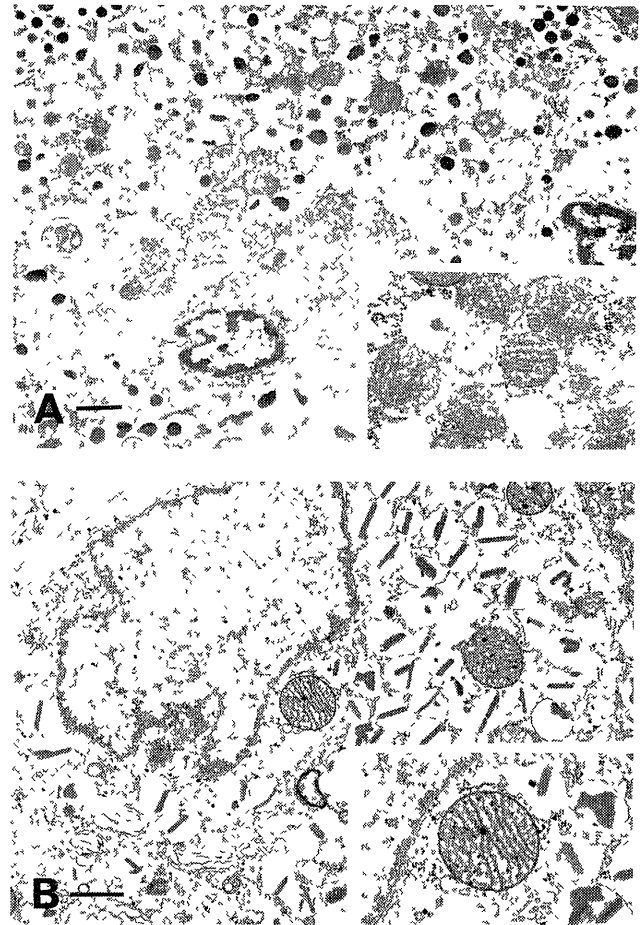


Fig. 2. (A) Electron micrograph of B cells in a pancreatic graft stored for 96h at 4 °C in UW solution (Group II) $\times 6,000$ Inset Slight swelling of mitochondria and a decrease in the number of cristae $\times 12,000$ (B) B cells of the graft at 6h after transplantation $\times 6,000$ Inset Clear cut cristae can be seen The number of cristae has increased and their arrangement is regular $\times 12,000$

cristae showed an increase (Fig 1b)

In the 96 hours group, mitochondrial swelling and a decrease in the number of cristae were seen in the islets, and the cristae could not be visualized clearly (Fig 2a) At 6 hours after autotransplantation, the outlines of the cristae became sharper and their number showed an increase (Fig 2b)

The mitochondria of acinar cells in the 72 hours group appeared to be condensed and featured an electron-dense matrix Their cristae were fragmented, shortened, or swollen (Fig 3a) At 6 hours after autotransplantation, the mitochondrial matrix looked normal and the arrangement of the cristae had recovered (Fig 3b)

In the 96 hours group, some of the mitochondria showed marked enlargement, a decrease in matrix density, and disordered arrangement of the cristae (Fig 4a), while others showed condensation (Fig 4b) By 6 hours after autotransplantation, the density of the mitochondrial matrix

had returned to normal and the arrangement of the cristae had recovered (Fig 4c)

The endothelial cells of the capillaries adjacent to the B cells were virtually normal in the 72 hours group and their organelles were well preserved (Fig 5a) At 6 hours after autotransplantation, the organelles of these cells remained intact and the basement membrane was unchanged (Fig 5b) In the 96 hours group, however, the basement membrane was damaged and there was an increase of cytoplasmic electron density in the endothelial cells (Fig 6a) At 6 hours after autotransplantation, these abnormalities had not been reversed (Fig 6b)

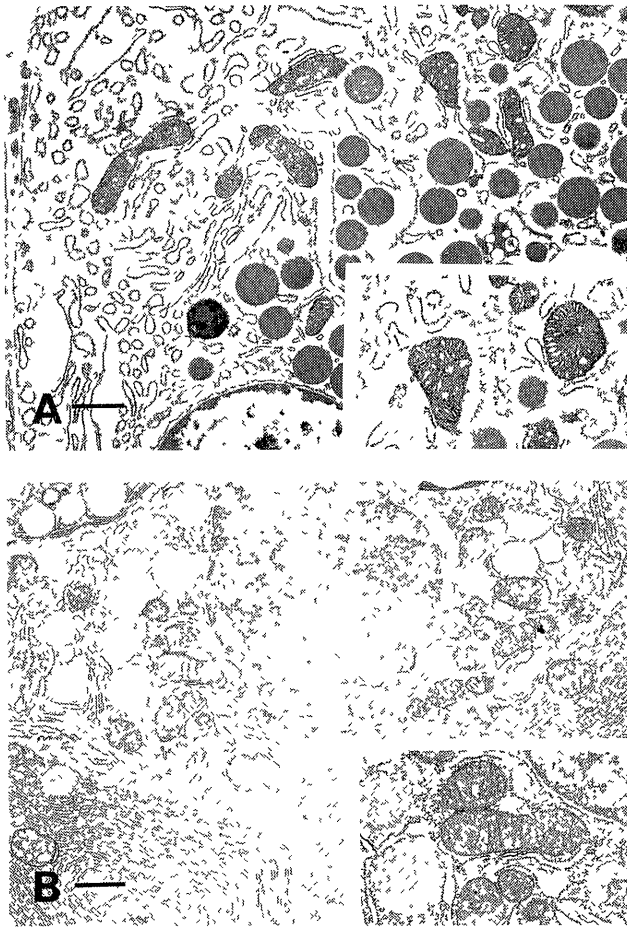


Fig. 3. (A) Electron micrograph of acinar cells in a pancreatic graft stored for 72h at 4 °C in UW solution (Group I) $\times 6,000$ Inset The mitochondria appear very electron-dense, and the cristae are fragmented, short, or swollen $\times 12,000$ (B) Acinar cells of the graft at 6h after transplantation $\times 6,000$ Inset The structure of the mitochondria is now almost normal $\times 12,000$

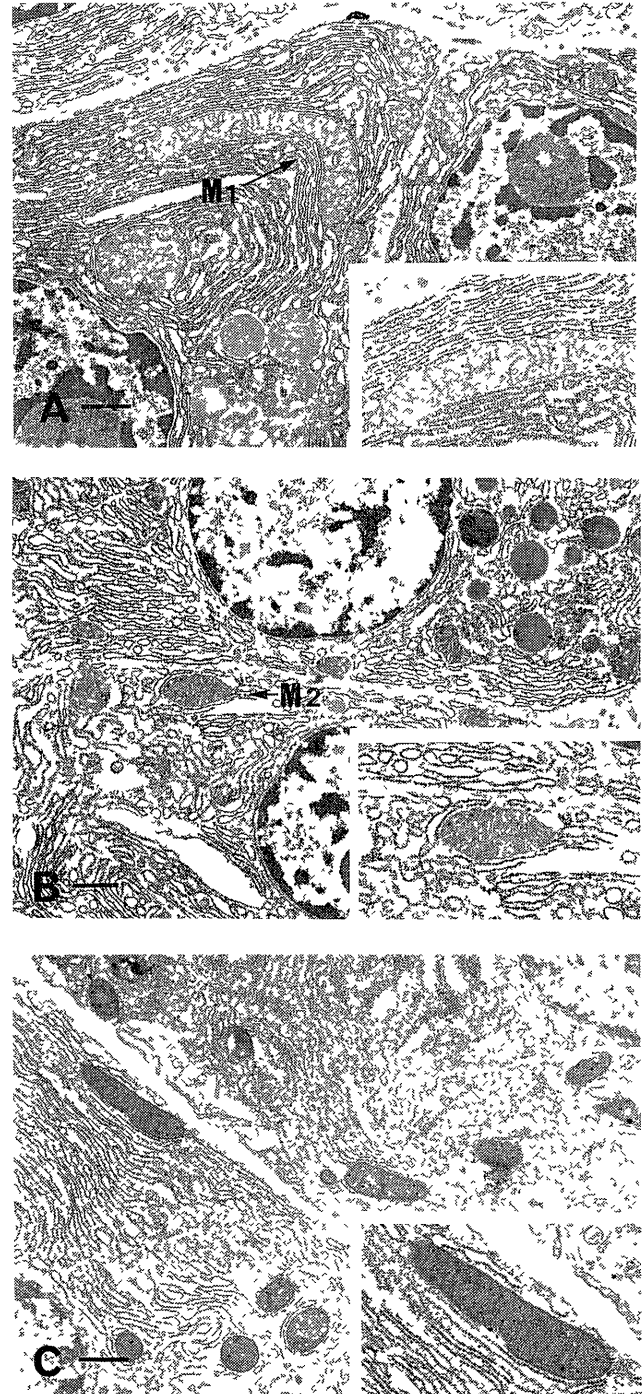


Fig. 4. (A, B) Electron micrographs of acinar cells in a pancreatic graft stored for 96h at 4 °C in UW solution (Group II) $\times 6,000$ Inset A some mitochondria [M1] showed marked enlargement, with a decrease in the electron density of the matrix and irregular arrangement of the cristae $\times 12,000$ Inset B Other mitochondria showed condensation [M2] $\times 12,000$ (C) Acinar cells of the graft at 6h after transplantation $\times 6,000$ Inset C The swelling or the electron-dense matrix of the mitochondria have returned to normal $\times 12,000$

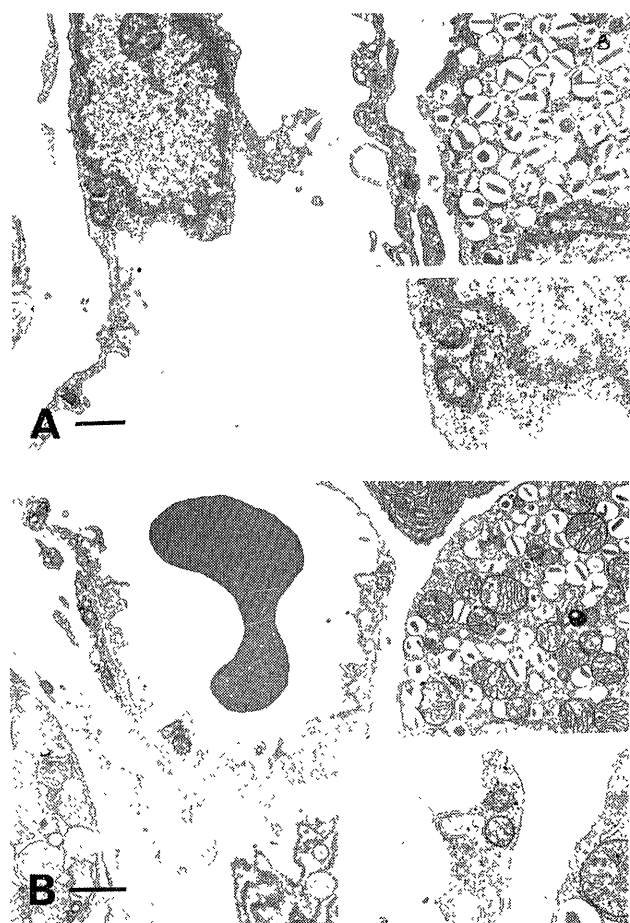


Fig. 5. (A) Electron micrograph of endothelial cells in an islet of a pancreatic graft stored for 72h at 4°C in UW solution (Group I). $\times 6,000$. Inset: The organelles of the endothelial cells and the endothelial lining were preserved well $\times 12,000$. (B) The islet endothelial cells at 6h after transplantation. $\times 6,000$. Inset: The cellular organelles are in good condition. $\times 12,000$.

Discussion

According to the International Pancreas Transplantation Registry Report of 1989, there was no difference in 1-year viability of the transplanted pancreas when the duration of cold ischemia was less than 24 hours or more than 24 hours (9). The viability of the preserved pancreas can be evaluated on the basis of endocrine function (10-11) or by ultrastructural observation of the mitochondria, which are directly related to cell function and survival (12). Our study demonstrated that pancreatic grafts preserved for 96 hours in cold UW solution could function well enough to maintain fasting plasma glucose level, although impairment of glucose tolerance was noted. The ultrastructural changes of the mitochondria during cold preservation were more severe in the acinar cells than in the islet B cells.

Caldwell *et al.* (13) demonstrated in their experiments on rat livers that cellular damage during preservation and

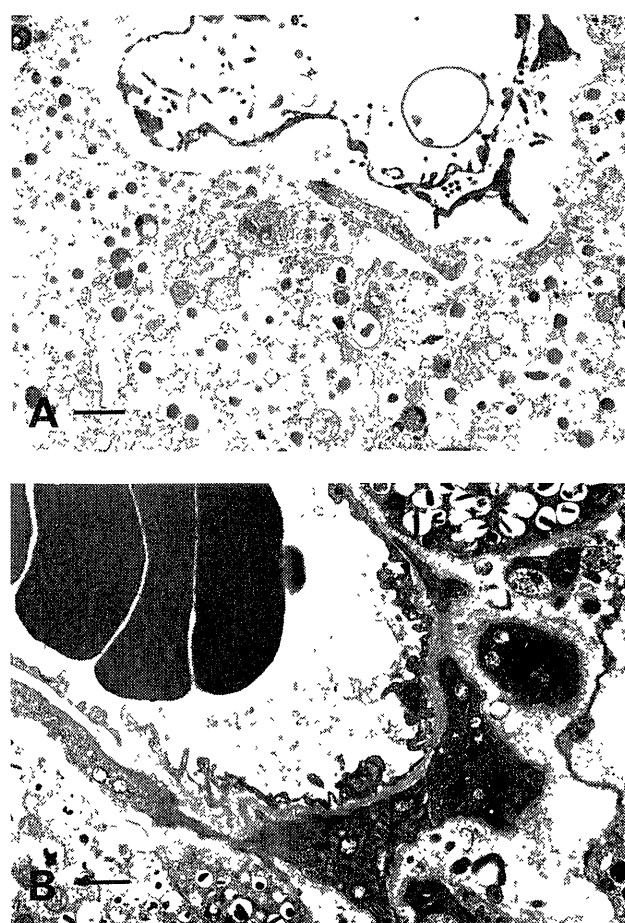


Fig. 6. (A) Electron micrograph of endothelial cells in an islet of a pancreatic graft stored for 96h at 4°C in UW solution (Group II). The endothelial cells had a high electron density and the endothelial lining had lost its continuity. $\times 6,000$. (B) Islet endothelial cells of the graft at 6h after transplantation. The endothelial cells have not recovered from the damage that developed during storage. $\times 6,000$.

reperfusion affected the nonparenchymal cells earlier than the parenchymal cells, and was especially prominent in the vascular endothelial cells. In our study, however, the vascular endothelial cells adjacent to the B cells retained normal organelles in the 72 hours group, although the basement membrane became irregular and the cytoplasm of the endothelial cells became more electron dense after 96 hours. This increase in electron density probably indicated degeneration and necrosis of the endothelial cells. Endothelial cells become edematous, which in turn disturbs the microcirculation, in response to the change from a high osmotic pressure environment during cold preservation to a low osmotic pressure environment on sudden exposure to warm circulating blood after transplantation. The ultrastructural damage to the endothelial cells in grafts preserved for 96 hours did not recover after graft reperfusion.

It has been reported that fibrin is deposited at sites where the microcirculation is disturbed by cold ischemia, predis-

posing to thrombosis (14). Thrombosis is a frequent complication of pancreatic transplantation (15), with venous thrombosis generally occurring within 48 hours and arterial thrombosis from 2 to 7 days after transplantation (16-17). As the causes of such thrombosis, a decrease of blood flow through the splenic artery and vein due to splenectomy, and platelet activation within the blind-ending splenic artery and vein have been suggested (18). With long-term cold storage of grafts, irreversible ischemic damage of the vascular endothelial cells will progress and their capacity to produce prostacyclin after reperfusion will markedly decrease (19).

Arterial thrombosis in the two dogs in the 96 hours group appeared approximately 2 weeks after autotransplantation. Microcirculatory disturbance due to irreversible degeneration of the endothelial cells following long-term cold preservation may have led to retrograde spread of arterial thrombosis in these animals. In addition, impairment of glucose tolerance in the 96 hours group may also have been associated with local disturbance of the microcirculation to the islets.

In conclusion, pancreatic storage for 72 hours has little effect on graft viability or function, while storage for 96 hours causes definite impairment.

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